WORKSHOP REPORT

Immunobarriers for Pancreatic Islet Transplantation

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Washington, DC

Sponsored by

The National Institutes of Health, U.S. Department of Health and Human Services
National Institute of Diabetes and Digestive and Kidney Diseases
National Institute of Biomedical Imaging and Bioengineering
and
The Juvenile Diabetes Research Foundation International

INTRODUCTION

A workshop entitled “Immunobarriers for Pancreatic Islet Transplantation” was convened in Washington, DC, on March 30-31, 2004, to review the state of the art in barrier material for tissue immunoisolation with the emphasis on pancreatic islet transplantation and other cell therapies for the treatment of diabetes. The workshop was sponsored by the National Institute of Diabetes and Digestive and Kidney Diseases, the National Institute of Biomedical Imaging and Bioengineering, and the Juvenile Diabetes Research Foundation International, and was chaired by Dr. Michael Lysaght of Brown University. Participants were invited from academia and industry, and included biomedical engineers, immunobiologists, cell biologists, diabetologists, and transplant surgeons. The meeting was organized to provide a forum for exchange of the most recent data and the latest insights and perspectives on the biomaterial components of what is commonly termed “the bioartificial pancreas”. It was strongly felt that a high priority should be placed on research into immunobarriers for pancreatic islet transplantation, and that a successful strategy would greatly enhance the clinical outcome. The meeting served to identify opportunities and barriers to progress Chief among these was the need for a clearer understanding of the mechanisms of both rejection and survival of encapsulated tissue, and less emphasis upon show-and-tell survival experiments in relatively compliant rodent models. Interdisciplinary teams with strong capabilities in islet-cell biology, membrane transport, biomaterials, immunology, etc., are required to achieve success in this field.

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EXECUTIVE SUMMARY

Islet Encapsulation--Goals and Strategies

The ultimate goal for all diabetes research is to generate an effective cure for diabetes, both type 1 and type 2 forms of the disease, with a target of glucose normalization at least equivalent to that achieved in the Diabetes Control and Complications Trial (DCCT). A current strategy that shows some success and a great deal of promise is islet replacement via transplantation of allogeneic cadaver pancreatic islets into an immunosuppressed recipient. This approach is constrained by the limited number of available pancreases, currently about 2000 per year, and by the side effects of pharmacological immunosuppression, which generally exceed the morbidity associated with the secondary effects of diabetes. There are alternative approaches under development to provide a more dependable supply of insulin-secreting tissue. These include the use of xenogeneic islets (e.g., porcine) or beta cell replacements engineered from immortalized beta cell lines or other cell types to secrete insulin in response to glucose and other nutrient secretagogues. Regardless of the source, it is expected that most of these potential cell therapies would require immunosuppression or immunoisolation to avoid graft rejection. A possible exception might be islets derived from autologous adult stem cells, but even this tissue might require protection from the host immune system in order to prevent recurrence of type 1 diabetic autoimmune destruction. Important progress is being made in tolerance and strategies to block autoimmuner rejection, but it is likely that the **clinical application of cell therapy for diabetes will depend on immunisolation as an enabling technology.**

The ultimate goal of islet encapsulation is increased islet graft survival and function following transplantation. Enough is known about the different components of the immune system at this time to allow researchers to conclude that complete immunoisolation may be difficult, whereas immunoprotection might be clinically useful and more easily achieved. All polymers meant to encase and shield islets from direct cell-cell contact must still be permeable to oxygen, nutrients, ions, insulin and other small signaling hormones. Indirect immune mechanisms are mediated by small molecules that can easily cross any such barrier. However, immune destruction of cells is a dose-dependent phenomenon, and it is a reasonable goal to reduce the quantity of pathogenic permeants below the level of gross cytotoxicity.

Islet grafts, whether encapsulated or not, must form a peri-vascular system quickly to ensure adequate oxygen and nutrient delivery for survival. Fibrotic or scar material surrounding the capsule may frustrate this goal, either by distancing capillaries too far from the islets or by presenting a physical barrier to transport from the capillaries to the islets. Fibrosis must be prevented, and angiogenesis promoted, if encapsulated islets are to become a viable therapy for diabetes. Proposed strategies to address these problems include short term treatment with angiogenic factors or other drugs embedded in the encapsulation material, or the use of biodegradable capsules.

State of the Art in Immunobarriers

The participants described a field which is very much at an inflection point. The paradigm that has guided design and development for the past three decades was the idea of an ideal barrier which could isolate islets from host immunoglobulin while permitting the passage of oxygen, glucose, and insulin. This was presumed to provide sufficient immunoprotection. It is increasingly clear that even were it obtained, this strategy is unlikely to be sufficient. Furthermore, it is now appreciated that encapsulated cells may succumb to a lack of oxygen, itself a consequence not of membrane impermeability, but of pericapsular inflammation caused by either material bioincompatibility or by an immune response of antigens shed from encapsulated cells. Nitric oxide from this same pericapsular inflammation may mediate cell destruction and no size selective membrane barrier can reject nitric oxide while simultaneously permitting passage of insulin.

Virtually all barrier materials must be sufficiently biocompatible so as not to invoke either a fibrotic or inflammatory response when implanted, even in the absence of foreign cells, in an immunocompetent host. Beyond this, material requirements vary significantly between allograft and xenograft islets. Allografts appear to require only a barrier which prevents cell-cell contact between graft and host in order to prevent rejection. Therefore, the significant issue for allograft immunobarriers is not to keep antibodies out of the capsule, but to permit sufficient inward transport of oxygen and nutrients required for cell survival and function. Xenografts are far more complex: soluble antigens leaching from the capsule are capable of provoking a local inflammatory response, which damages encapsulated cells by a variety of mechanisms even in the absence of any host antibodies slipping past the barrier into the capsule. It seems likely that additional interventions, beyond improved barrier selectivity, will be required for successful immunoisolation of xenogeneic cells.

Symposium participants suggested that past research in this field, largely funded by the provate sector, has been “outcomes driven” to an extent which excluded support for basic science. The literature contains reports of several hundred or more experiments in which the survival of encapsulated islets has been evaluated in rodent, canine, and non-human primate models. Some results have been encouraging, especially in rodents. Most attempts to treat diabetes in large animal models have been unsuccessful. Unfortunately, failure mechanisms have rarely been sorted out and participants at the workshop felt that careful investigation and documentation of the reasons for failure in unsuccessful experiments was an essential requirement for future progress.

Just as the immunoisolation paradigm that has governed early islet encapsulation research is outdated, the field has yet to incorporate novel approaches based upon recent insights and new technologies. Examples of such new ideas include genetically engineered or cloned islets with reduced immunogenicity, nanofabricated barriers or assemblies with tightly controlled pore size and optimal special distribution of cells, oxygen transport enhancers within the capsule material, use of transient immunosuppression in the period following implantation, and local release of anti-inflammatory agents from the capsule (in analogy with drug releasing stents). Successful development of such new approaches,

along with a clear understanding of their impact, is expected to be a highly fruitful area for future research.

Common Themes and Critical Insights
The following themes were found in multiple presentations or were particularly prominent in discussion.

1. The original paradigm of immunoisolation was to impose a semipermeable barrier between islet and host. Oxygen secretagogues and insulin would pass through this barrier, while antibodies would not. This has now been replaced by a much more complex and subtle understanding. Cell death inside immunobarriers, when it occurs, may be mediated by a) very small molecules such as nitric oxide or b) intracapsular hypoxia, itself a possible consequence of pericapsular inflammation caused by either material bioincompatibility or by an immune response to soluble antigens.

2. The two most common classes of biomaterials used as immunobarriers are thermoplastic polymers (e.g., polysulfone) and marine hydrocolloids (e.g., alginate). Much more work is needed to characterize the permselectivity and implant-relevant biocompatibility of both types of materials. Thermoplastic polymers are reasonable standard, but depending upon the details of fabrication and formulation, membranes made from the same material can differ enormously in both biocompatibility and transport. The case with alginate is further complicated by the inherent variability of this naturally occurring material. Most investigators agree that alginate used to fabricate capsules must be standardized and purified, but each group has its own definitions of standards and its own requirements for purity.

3. Success in rodents is not predictive of success in larger animal models. This simple fact should be borne in mind when evaluating the potential contribution of new technologies which have only been demonstrated in rodent models.

4. An inflammatory response may be raised even to necrotic *autologous* tissue. Therefore, even an encapsulated autograft may lead to an immune response if the capsule design or transplantation protocol allows the tissue to become hypoxic, and then necrotic.

5. In most investigators' experience with encapsulation, cell death begets further cell death. It is therefore important to avoid, even transiently, circumstances in which the number of encapsulated cells exceeds the carrying capacity of the capsule. Therefore, the function of fewer, well oxygenated islets may exceed that of a much larger poorly perfused cohort.

6. Several participants advocated a "learn to walk before attempting to run" approach, and suggested that immune barriers be developed and evaluated in progressive fashion with staged goals of increasing complexity. One format

would be to advance from transplantation of functionally autologous islets, to allogeneic islets, then finally to xenogeneic islets. An alternative approach would be encapsulation first in immunocompetent hosts, then in syngeneic hosts, and finally in donor-recipient combinations more reflective of clinical reality.

7. Implantation of additional helper cell types along with the endocrine tissue, has been pursued. Specifically, Sertoli cells embedded along with the pancreatic islets are thought to enhance engraftment.

8. A number of clinical trials of cells transplanted behind an immunoisolation barrier, largely in the central nervous system, have demonstrated the capacity of these barriers to preserve the viability and function of encapsulated cells for periods approaching one year. Such results provide proof of principle for the technology, but they also point out the challenges. Success is most readily achieved in applications involving a relatively small number of cells derived from a dividing cell line and implanted into a liquid space, in an immunopriviliged site (e.g.,eye or CNS).

9. Specific knowledge regarding the life cycle of the islet in capsules, either in vitro or transplanted into animals, is lacking. This knowledge may help researchers understand the mechanisms leading to functional failure and cell death.

Recommendations

Enhanced resources will increase the likelihood of success in this field. It is important to pursue all avenues related to immunoisolation in order to ensure success of islet transplantation. The following resources would stimulate and improve science in the area of immunoisolation.

1. Means by which investigators could obtain porcine islets for encapsulation research.
2. Means by which investigators could have access to large animal models, in particular non-human primates. To achieve this goal, an appropriate large animal model should be developed.
3. Means by which the components of this multidisciplinary field could work together, including meetings, a consortium, a newsletter, etc.
4. Having a meeting to define reference standards for the field and the establishment of accessible reference standards.
5. Tools and technologies (imaging) for assessment of islet physiology and function within capsules.
6. Facility sponsored through FDA/NIST for assessing the properties of alginate (purity, structure, etc.).

Concluding Perspective

It is the sense of this workshop that successful development of immunoprotective barriers is prerequisite to widespread application of cell therapy to diabetes. This will depend on highly integrated teams of researchers from the fields of transplantation biology, islet biology, physiology, immunology, polymer chemistry and bioengineering. There is a need for basic biology studies to uncover whether sufficient nutrients are delivered, to detail the complex nature of the host defense, and to define the mechanisms by which materials fail in the transplanted environment, and by which the islets lose function and die.

MEETING AGENDA

MEETING ABSTRACTS

CONFERENCE PARTICIPANTS

ADDENDUM: SALIENT QUESTIONS RAISED DURING DISCUSSION

During the discussion, the following questions were raised that require experimental research to answer them:

1. Is necrosis a major signal to elicit an inflammatory response?
2. Are host cells (immune cells, fibrotic cells) effectively suffocating the encapsulated islet by using available oxygen and nutrients?
3. What are the appropriate properties for the best achievable immunoisolation material?
4. What mechanisms make up the indirect pathway of immune destruction?
5. What are the failure mechanisms for encapsulated islets?
6. What benefit, outside that of an immunobarrier, is conferred by a capsule to an islet, given that islets appear to do better in vitro once encapsulated?
7. How do capsules of various materials change in time in the in vivo environment?
8. Define the properties of different sites for capsule implementation—portal vein vs intraperitoneal.
9. What is the optimal dosage of islets to achieve optimal engraftment? Optimal function?